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Effect of Hepatitis C Virus (HCV) on Hemoglobin, Blood Cells and Random Blood Glucose Levels among Serologically Positive HCV Patients

Mohammad Asaduzzaman[*1], Salim Reza Bappy[1], Babry Fatema[1], Shaila Haque[1], Mohammod Johirul Islam[2], Md. Asaduzzaman Sikder[2], Sharmin Aktar[2], Marina Khatun[2], Farha Matin Juliana[3], Mohammad Sayful Islam[4]

[1] *Primeasia University, Department of Biochemistry, Banani, Dhaka-1213, Bangladesh*
[2] *Department of Biochemistry and Molecular Biology, Mawlana Bhashani Science and Technology University, Santosh, Tangail-1902, Bangladesh*
[3]*Department of Biochemistry and Molecular Biology, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh*
[4]*Department of pharmacy, Mawlana Bhashani Science and Technology University, Santosh, Tangail-1902, Bangladesh*
Corresponding Author: Mohammad Asaduzzaman

Abstract: *Chronic Hepatitis C Virus (HCV) infection is associated with an increased risk of developing insulin resistance (IR) and type 2 diabetes (T2D). The study population consisted of 38 patients (63.2% anti-HCV antibody-positive adult male and 36.8% anti-HCV antibody-positive adult female). This study examines the predominance of anemia (less RBC and hemoglobin) and random blood sugar (RBS) in HCV infected patients. Along with anemia and RBS it is observed that 23.7% HCV infected patients are with less platelet count than reference value. Differential count of WBCs such as Neutrophil, Monocytes, Lymphocytes and Eosinophil are found significantly associated with HCV positive individuals both male and female. HCV positive male individuals have less likelihood (19 out of 24) to develop anemia than female (13 out of 14). Here it is found that 79.2% and 87.5% male and 92.9% and 92.9% female HCV patients suffer from anemia due to less hemoglobin content and less RBC count respectively. The prevalence of high blood sugar is higher in HCV infected patients examined. 28.95% male and 18.42% female HCV patients suffer from hyperglycemia.*

Keywords: *HCV, Glucose, Hemoglobin, RBC, WBC, Platelet.*

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I. Introduction

The Hepatitis C virus (HCV) is a blood borne virus causing both acute and chronic liver diseases. HCV infection in the liver triggers the immune system, which leads to inflammation, liver cirrhosis as well as hepatocellular carcinoma. Approximately, 170 million people worldwide are chronically infected with the HCV. The virus persists in the liver in about 75% to 85% of those initially infected. Early on chronic infection typically has no symptoms. Over many years however, it often leads to liver disease and occasionally [(1)].

HCV is a hepatotropic virus [(2)] mainly affects the liver, but also several tissues outside the liver have been reported to be involved, resulting in a wide spectrum of extrahepatic manifestations [(3, 4, 5)]. During the last decade, it has been hypothesized that diabetes could be one more of these extrahepatic conditions attributable to HCV infection. This raises the intriguing question of whether the rise in HCV infection is contributing to the increasing prevalence of type 2 diabetes.

Hepatic iron overload is one of the pathophysiologic features of hepatitis C virus (HCV)-associated chronic liver disease, even though the level of hepatic iron content is not extremely high [(6)]. Anemia with iron overload is a condition of chronic HCV infection that impairs the normal transport of iron in cells. Iron is an essential component of hemoglobin, which is the substance that red blood cells use to carry oxygen to cells and tissues throughout the body. In this condition, red blood cells cannot access iron in the blood, so there is a decrease of red blood cell production (anemia) that is apparent at birth. The red blood cells that are produced are abnormally small (microcytic) and pale (hypochromic). Hypochromic microcytic anemia with iron overload can lead to pale skin (pallor), tiredness (fatigue), and slow growth. In hypochromic microcytic anemia with iron

overload, the iron that is not used by red blood cells accumulates in the liver, which can impair its function over time. The liver problems typically become apparent in adolescence or early adulthood [(7)].

HCV infection has been associated with immunologic disorders such cryoglobulinemia, glomerulonephritis, thyroiditis, and Sjöegren syndrome [(3, 4, 5)]. It might be then thought that HCV could trigger an immune reaction against the β-cell that leads to diabetes. In this case, a possible pathogenic mechanism could be molecular mimicry, because HCV shares regional amino acid homology with GAD autoantibody (GADA), one of the main islet cell antigens [(8)].

Besides hepatic complications, chronic HCV infection is also associated with several extra-hepatic manifestations including thrombocytopenia. Thrombocytopenia in chronic HCV infection is a major problem, particularly in patients with advanced liver disease. The risk of serious bleeding with severe thrombocytopenia can prevent invasive procedures including biopsies for staging [(9)]. Thrombocytopenia can also complicate bleeding manifestations such as variceal bleeding. It may impede the initiation and continuation of antiviral therapy, potentially decreasing the probability of successful HCV treatment [(10)].

Therefore, this study was designed to assess blood glucose, hemoglobin, red blood cell (RBC), white blood cells (neutrophils, lymphocytes, monocytes, eosinophils and basophils) and platelet status in chronic HCV-infected patients.

II. Materials and Methods

Materials

Study Population:

38 Hepatitis C Virus infected patients.

Sample collection and period:

Samples were collected in Dhaka Central International Medical College and Hospital, Bangabandhu Sheikh Mujib Medical University (BSMMU), Popular Diagnostic Center, Islami Bank Hospital, Dhaka, Bangladesh during January 2017 to April 2017.

Methods

Red Blood Cell (RBC), White Blood Cell (WBC), Platelet (PLT) Count:

The Sysmex XT-2000i Automated Hematology Analyzer is used to count RBC, WBC and PLT which utilizes the power of fluorescent flow cytometry and hydrodynamic focusing technologies. Using a unique, diode laser bench, Sysmex fluorescent flow cytometry provides the sensitivity needed for measuring and differentiating cell types in whole blood and body fluid samples.

a) RBC and PLT Count:

Sysmex analyzer XT-2000i uses the DC sheath flow detection method [(11)] to count red blood cells and platelets, RBC and PLT. A portion of blood is separated from the aspirated whole blood and mixed with the diluent in a pre-set ratio.

Of this dilution a defined amount is sent to the detection chamber and passed through a small opening, known as the aperture. There are also electrodes on each side of the aperture-and direct current passes through these electrodes. The direct current resistance between the electrodes changes as blood cells suspended in the diluent pass through the aperture. This resistance causes an electrical pulse change proportional to the size of the blood cell. These electrical data are converted into graphical displays of volume distribution curves, or histograms.

Once the cells leave the sample nozzle exit they are surrounded by a sheath flow of diluent. Here, they are aligned and moved to the centre of the orifice. This reduces interference errors and the possibility of abnormal cell pulse detection, which could be caused by cells passing through the transducer off-centre. As soon as the cells have passed the orifice, they are seized by another, inverse flow and immediately led to the drain. This prevents renewed circulation and a change in the platelet count.

b) WBC Count:

Sysmex analyzer XT-2000i counts WBC by using the combination of side scatter (cell complexity), forward scatter (cell size) and fluorescence (DNA and RNA concentration) of nucleated cells provides a concise and precise image of each detected peripheral blood cell. This 3-dimensional blood cell analysis provides unique accuracy and precision. Fluorescence labeling of peripheral blood cells is a milestone for the routine leukocyte differential. Fluorescent technology enables the XT to reliably differentiate normal WBC populations from abnormal WBC populations. The sensitivity of the unique application of fluorescent flow cytometry gives the lab a high level of confidence in reporting accurate WBC differentials, even on critical patient samples when the WBC count is low.

c) Hemoglobin Measurement:

Sysmex analyzer XT-2000i uses the SLS detection method [12, 13] to measure the content of blood hemoglobin. Sodium Lauryl Sulphate (SLS) is a surfactant which both lyses erythrocytes and rapidly forms a complex with the released hemoglobin. The product SLS-MetHb (Methemoglobin) is stable for a few hours and has a characteristic spectrum with maximum absorbance at 539 nm [14]. The complex obeys Beer-Lambert's law so there is a precise linear correlation between Hb concentration and absorbance of SLS-MetHb. The method simply involves mixing 25µL of blood with 5.0mL of a 2.08mmol/L solution of SLS (buffered to pH of 7.2), and reading absorbance at 539 nm. The results of ctHb (total hemoglobin concentration) by the SLS-Hb method have been shown to correlate very closely (r=0.998) with the reference HiCN (hemoglobincyanide) method [13]. The method has been adapted for automated hematology analyzers and is as reliable in terms of both accuracy and precision as automated HiCN methods [13, 15, 16]. A major advantage is that the reagent is non-toxic. It is also less prone to interference by lipemia and increased concentration of leukocytes [13].The long-term instability of SDS-MetHb precludes its use as a standard so the method must be calibrated with blood whose ctHb has been determined using the reference HiCN method.

Random Blood Sugar:

For random blood sugar investigation blood was collected from a vein, typically from the inside of our elbow or from the back of our hand. This investigation was carried out by manual and automated methods. 2ml of Venus blood was collected into BD Vacutainer™ Plastic Blood Collection Tubes with a specific amount of Sodium Fluride/Na2 EDTA. Then wait for few minute. This blood sample was centrifuged for the separation of plasma from blood. Plasma was collected and put on the sample cup for test. For manual method 1ml of (glucose oxide) sugar reagent was taken into a sample test tube and added 10µl blood plasma from the sample cup; and wait for 10minutes. Absorbance was taken after 10minutes by semi-auto analyzer. For automated method aliquot of the blood plasma in the sample cup was put into the analyzer to get the random blood sugar concentration [17].

III. Results And Discussion

Study population consisted of 38 patients (63.2 % anti-HCV antibody-positive male and 36.8 % anti-HCV antibody-positive female) divided into two groups. The first group consisted of patients with HCV positive male 24, and the second group consisted of patients with HCV positive female 14.

Table 1: Normal and anemic condition of HCV positive male and female as per RBC count

	RBC			Total in 100%
	Reference Value [18]	Normal condition %	Anemic condition %	
Male	4.3 to 5.9 million cells per microliter	3 (12.5)	21 (87.5)	24 (63.2)
Female	3.5 to 5.5 million cells per microliter	1 (7.1)	13 (92.9)	14 (36.8)

Sysmex analyzer XT-2000i analyzer uses the DC sheath flow detection method.

Table 1 represented that the mean RBC by categorizing anemic and non-anemic condition for the HCV positive male and female patients. Among 38 patients 87.5% anti-HCV antibody-positive male and 92.9% anti-HCV antibody-positive female) were assessed as anemic due to less count of RBC. And 12.5% anti-HCV antibody-positive male and 1% anti-HCV antibody-positive female were non-anemic due to sufficient count of RBC among HCV infected patients.

Table 2: Platelet count among HCV infected patients

Name of Cells	Reference value [18]	Less than normal count(%)	Normal count(%)	Above the normal count (%)
Platelet	150000-450000 per microliter	9 (23.7)	29(76.3)	0 (0)

Platelet count below the normal range was 23.7%; within the normal range was 76.3%. Sysmex analyzer XT-2000i uses the DC sheath flow detection method.

No any HCV positive individual's platelet count found above the normal range but below the normal range was found for 23.7% and normal range was found among 76.3% patients.

Table 3: Normal and anemic condition of HCV positive male and female as per Hemoglobin content

	Hemoglobin			Total in 100%
	Reference Value [18]	Normal condition %	Anemic condition %	
Male	2.1-2.7 mmol/L	5 (20.8)	19 (79.2)	24 (63.2)
Female	1.9-2.5 mmol/L	1 (7.1)	13 (92.9)	14 (36.8)

Sysmex analyzer XT-2000i uses the SLS detection method.

Table 3 represented that the mean hemoglobin by categorizing anemic and non-anemic condition for the HCV positive male and female patients.

Among 38 patients 79.2% anti-HCV antibody-positive male and 92.9% anti-HCV antibody-positive female) were assessed as anemic due to less content of hemoglobin. And 20.8% anti-HCV antibody-positive male and 7.1% anti-HCV antibody-positive female were non-anemic due to sufficient content of hemoglobin.

Table 4: WBC distribution among HCV infected patients

	WBC					
Valid	Parameters	Frequency	Percentage (%)	Gender	WBC Reference Value [18]	Percentage (%)
	Normal Count	16	42.1	Male	4,500 to 11,000 WBC per microliter	26.32
				Female		15.79
	Higher than normal Count	22	57.9	Male		36.84
				Female		21.05
	Total	38	100.0	Total		100.00

WBC count was found normal in 42.1% patients and 57.9 % were above the reference standard. 26.32% male and 15.79% female found normal; and 36.84% male and 21.05% female found higher than normal count. Sysmex XT-2000i analyzer; side scatter, forward scatter and fluorescence method.

Table 4 presented that among 38 HCV positive patients WBC count for 42.1% were found normal that means within the reference standard and 57.9 % were above the reference standard.

Table 5: Distribution of WBC subpopulations such as Neutrophil, Monocyte, Eiosiniphil, and lymphocyte among HCV infected patients

Name of ells	Reference Value [18]	Bellow than Normal count(%)	Normal count(%)	Above Than Normal count (%)
Neutrophil	45-62% of WBC	1(2.6)	16(42.1)	21(55.3)
Monocyte	3-7% of WBC	4(10.5)	34(89.5)	0 (0)
Eosinophil	1-3% of WBC	12(31.6)	22(57.9)	4(10.5)
Lymphocyte	16-33% of WBC	5 (13.2)	21 (55.3)	12 (31.6)

Neutrophil, monocyte, eiosiniphil and lymphocyte counted below the normal range were 2.6%, 10.5%, 31.6%, and 13.2%; within the normal range were 42.1%, 89.5%, 57.9%, 55.3%; and above the normal range were 55.3%, 0%, 10.5%, 31.6% respectively. Sysmex XT-2000i analyzer; side scatter, forward scatter and fluorescence method.

Table 5 described neutrophil, monocyte, eisoniphil and lymphocyte counts among 38 HCV positive individuals in respect of below the normal range, within normal range and above the normal range.

Neutrophil counted below the normal range, within normal range and above the normal range were 2.6%, 42.1% and 55.3% respectively. Monocytes counts below the normal range and within normal range were 10.5% and 89.5% respectively. 31.6%, 57.9% and 10.5% of the infected individuals showed that the eosinophil count below the normal range, within the normal range and above the normal range respectively. Lymphocyte counted below the normal range, within normal range and above the normal range were 13.2%, 55.3% and 31.6% respectively.

Table 6: Assessment of random blood sugar among HCV infected patients

	Random Blood Sugar					
Valid	Parameter	Frequency	Percentage (%)	Gender	Reference Value [19, 20, 21]	Percentage (%)
	Hypoglycemia	1	2.6	Male	Less than reference value	0.00
				Female		2.63
	Normal	19	50.0	Male	3.9-7.8 mmol/L	34.21
				Female		15.79
	Hyperlycemia	18	47.4	Male	More than reference value	28.95
				Female		18.42
	Total	38	100.0			100.00

2.6% patients were found with hypoglycemic, 50.0% was found with normal condition and 47.4% patients were found with hyperglycemic. 34.2% male were in normal range and 28.9% were in hyperglycemic conditions. 2.6% HCV female patients were in hypoglycemic condition and 18.4% were with hyperglycemic condition and others were in normal condition.

Table 6 stated that among 38 HCV patients 2.6 % individuals were in hypoglycemic condition, 50.0% patients were in normal condition and 47.4% individuals were in hyperglycemic condition.

HCV affects glucose metabolism via triggering an immune reaction against the β-cell of islet that leads to diabetes. Anemia with iron overload is a condition of chronic HCV infection that impairs the normal transport of iron in cells. In this condition, red blood cells cannot access iron in the blood, so there is a decrease of red blood cell production (anemia). Hepatitis C infection, Anemia and Diabetes are chronic diseases with a high global prevalence of epidemic proportions. The association among Hepatitis C infection, Anemia and Diabetes have been substantiated by numerous studies in the past two decades. HCV infection triggers diabetes and anemia largely and this is already organized by many research. The HCV positive female has more risk to develop anemia than male. Mostly, Differential WBCs such as Neutrophil, Monocyte, Eosinophil, lymphocytes; and Platelets are significantly associated with HCV positive individuals both male and female. But, according to

hemoglobin analysis, HCV positive male individuals have less affinity to produce anemia than female. Our findings indicate that, Neutrophil, Lymphocytes, Monocyte, Eosinophil and Platelets are significantly associated with HCV positive individuals both male and female.

IV. Conclusion

This study has demonstrated a strong association between HCV and high blood sugar level, importantly; Anemia and Diabetes are common in HCV positive patients. Our study summarizes the prevalence of high blood sugar and anemia (less hemoglobin and RBC) is higher in HCV infected patients. Further prospective studies are needed that take the HCV viral load and degree of histologic liver damage for metabolic evaluation of glucose handling.

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